

Possible Involvement of Endogenous β -Endorphin in the Pathophysiological Mechanisms of Pichinde Virus-Infected Guinea Pigs¹ (43440)

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Abstract. Previously, we demonstrated that naloxone, an opiate antagonist, prolonged survival of strain 13 guinea pigs infected with Pichinde virus. Thus, endogenous opiates may be involved in the pathogenesis of this viral disease. To determine whether endogenous opiate levels were affected by Pichinde viral infection, β -endorphin concentrations in plasma and cerebrospinal fluid (CSF) of normal and infected strain 13 guinea pigs were measured by radioimmunoassay. Cerebrospinal fluid β -endorphin concentrations were 78.0 ± 13.2 pg/ml on postinoculation day (PID) 7, 59.0 ± 5.6 pg/ml on PID 12, and 58.8 ± 5.4 pg/ml on PID 14. These values were significantly higher than baseline levels of CSF β -endorphin: 30.8 ± 1.9 pg/ml. Plasma β -endorphin concentrations of infected animals increased significantly to 202.1 ± 17.9 pg/ml on PID 7 and to 154.2 ± 21.4 pg/ml on PID 12 from a mean baseline value of 84.2 ± 13.1 pg/ml. After a primer intravenous injection of β -endorphin (10, 15, or 30 μ g/kg), followed by constant infusion of β -endorphin (15, 45, or 90 μ g/kg·hr) to control noninfected guinea pigs, heart rate (except with the lowest dose) and mean blood pressure decreased markedly. Under these experimental conditions, concentrations of plasma and CSF β -endorphin increased simultaneously with different magnitude. Because both Pichinde viral infection and β -endorphin administration produced a similar trend of cardiovascular disturbances, leading to hypotension and bradycardia, increased concentrations of plasma and CSF β -endorphin may play a partial role in the pathophysiological mechanisms of Pichinde virus infection.

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The strain 13 guinea pig infected with Pichinde virus has been used as an animal model for studying pathophysiological mechanisms and treatment of arenavirus-induced Lassa fever in humans (1–4). Peters *et al.* (2, 4) and Liu (5) reported that cardiac functions of Pichinde virus-infected guinea pigs were depressed or impaired by postinoculation day

(PID) 7 throughout the disease course. Functional modifications of the heart in the infected animal were induced without significant histopathological changes. Furthermore, among many other organs examined, no virus could be demonstrated in the heart of Pichinde viral infection (3). These previous findings (2–5) suggest that the cardiac dysfunction during Pichinde virus infection may be a result of chemically induced organ dysfunction, which may be associated with deleterious actions of certain endogenous mediators.

Administration of β -endorphin into the brain or peripheral circulation of rats and dogs produces hypotension, bradycardia, and decreased coronary flow, as well as reductions of stroke volume and cardiac contractility (6–9). Physiological stress can also induce increased β -endorphin concentrations in plasma and cerebrospinal fluid (10, 11). Naloxone, an opiate antagonist, lessens the hypotension in endotoxemic (12), hemorrhagic (13), and spinal neurogenic shock in ani-

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mals (14). In human studies, Roberts *et al.* (15) observed that the stroke volume of patients with septic shock was improved by intravenous infusion of naloxone. In the naloxone-treated patients, inotrope and vasopressor requirements to maintain mean blood pressure were significantly lower than in those patients without naloxone treatment. Moreover, Liu *et al.* (16) found that the survival of Pichinde virus-infected guinea pigs was prolonged by daily subcutaneous injections of naloxone.

Guillemin *et al.* (17) reported that β -endorphin and ACTH are derived from a common precursor protein (β -lipotropin) and possess identical regulatory and release mechanisms. We demonstrated that the weight of adrenal cortex increased 80% in the Pichinde virus-infected guinea pigs, and plasma cortisol and aldosterone concentrations increased significantly (2, 18). Morphological and functional changes of the adrenal cortex postinoculation of Pichinde virus might be associated with an increased ACTH secretion from the pituitary. Thus, we hypothesized that more β -endorphin may be secreted from different sources, including brain and pituitary gland or other peripheral tissues, during Pichinde virus infection. Possibly, a linkage may exist between the neuroendocrine system and cardiovascular disturbances.

The main purpose of this study was to determine whether plasma and cerebrospinal fluid (CSF) β -endorphin concentrations were modified during Pichinde virus infection. Furthermore, the relationship between β -endorphin and hemodynamic changes in heart rate, as well as arterial mean, systolic, and diastolic blood pressures, were evaluated in control noninfected guinea pigs after constant intravenous infusion of β -endorphin at different doses.

Materials and Methods

Experimental Design. Fifty-nine male strain 13 guinea pigs, weighing 500–700 g, were allocated into three control and five Pichinde virus-infected groups. Cerebrospinal fluid and blood samples were withdrawn separately from 10 and nine control animals, respectively. An additional five control guinea pigs were intravenously infused with exogenous β -endorphin, and both blood and CSF samples were collected from the same animal.

Animals of infected groups were inoculated subcutaneously with 10^4 plaque-forming units of Pichinde virus (1–3). Cerebrospinal fluid was sampled on PID 7 for infected group 1 ($n = 9$), PID 12 for infected group 2 ($n = 6$), and PID 14 for infected group 3 ($n = 6$). Arterial blood was obtained on PID 7 from infected group 4 ($n = 6$) and on PID 12 for infected group 5 ($n = 8$).

Anesthesia and Surgery. The guinea pig was anesthetized intraperitoneally with sodium pentobarbital

(40 mg/kg for control and 25 mg/kg for sensitive, infected animals). The left common carotid artery was catheterized for collecting blood samples and measuring arterial blood pressures. Heparin was used as an anti-coagulant. The left external jugular vein was also cannulated for infusion of isotonic saline at 10 ml/kg·hr.

CSF Sampling. In anesthetized guinea pigs, cisternal punctures were made stereotactically, as previously described (19), by using an L-shaped, 23-gauge needle connected to 2 cm of polyethylene tubing (PE-50; i.d. = 0.58 mm, o.d. = 0.965 mm) fitted to a 1-ml glass syringe. The syringe and needle adapter were surrounded with crushed ice. Cerebrospinal fluid was collected for 3 min and was transferred from the syringe to a glass vial sealed with a tight cap. The glass vial with CSF sample was immediately frozen in liquid nitrogen to prevent further destruction of β -endorphin by proteinase. The same procedures of CSF withdrawal and freezing were repeated three times over 15 min at a 3-hr interval for 6 to 12 hr.

During the entire period of CSF collection, isotonic saline was infused intravenously into the guinea pig at 10 ml/kg·hr. Cerebrospinal fluid samples from the same animal were pooled. After CSF sampling was completed, the glass vial with CSF was taken from liquid nitrogen, and immediately placed in a boiling water bath for 10 min to abolish proteinase activity (20). The heat-treated CSF samples were stored at -70°C until radioimmunoassay of β -endorphin.

Plasma Preparation. Two milliliters of arterial blood were collected and transferred into glass tubes, each containing 20 μl of 0.25 M EDTA. Plasma was separated from blood cells by centrifugation at 760g at 4°C for 15 min and stored at -70°C .

Radioimmunoassay of β -Endorphin. Plasma and CSF β -endorphin concentrations were measured with radioimmunoassay kit (Incstar Corp., Still Water, MN). The measurement of β -endorphin consists of two major procedures: (i) extraction of β -endorphin from plasma and CSF samples, and (ii) measurement of the aluminum-adsorbed β -endorphin with radioimmunoassay. The method for calculating β -endorphin concentrations in plasma and CSF samples was based on a simultaneously determined standard curve of authentic β -endorphin. Various β -endorphin concentrations in standard plasma were plotted against the extent of bound radioactivities of β -endorphin with the specific antibody. With this kit, antibody to β -endorphin has been demonstrated to develop 5% cross-reactivity with β -lipotropin, but no cross-reactivity with other peptides (11).

About 1 ml of CSF was required for β -endorphin radioimmunoassay. When the CSF sample of any animal was less than 1 ml, CSF samples were pooled from two animals. There were eight CSF samples from the control group, seven samples from infected group 1, and six samples from both infected groups 2 and 3.

Intravenous Infusion of β -Endorphin

When mean arterial blood pressure was stabilized after surgery, a priming dose of β -endorphin (5, 10, 15, or 30 $\mu\text{g/kg}$) was injected intravenously into five guinea pigs and was followed by constant infusion of β -endorphin at 5, 15, 45, or 90 $\mu\text{g/kg}\cdot\text{hr}$, respectively. Four blood samples (1 ml each) were collected at 0, 10, 30, and 60 min after β -endorphin infusion. CSF samples were collected in two out of five guinea pigs 60 min after the β -endorphin primer injection (10 $\mu\text{g/kg}$) and followed by infusion at 15 $\mu\text{g/kg}\cdot\text{hr}$. We determined β -endorphin concentrations in these samples, but plasma β -endorphin concentration was too high to measure. Arterial blood pressures and heart rate were measured before and after β -endorphin infusion for the first 10 min without blood sampling.

Results

The mean CSF β -endorphin concentration in control strain 13 guinea pigs was 30.8 ± 1.9 pg/ml, which was significantly lower than plasma values (84.2 ± 13.1 pg/ml) (Fig. 1). Plasma β -endorphin concentrations of Pichinde virus-infected guinea pigs increased 140% on PID 7 and increased 100% on PID 12. Likewise, CSF β -endorphin concentrations of infected guinea pigs were also increased exceeding preinoculation values by 180% on PID 7 and 100% on PID 12 (Fig. 1). When virus-infected animals were near death on PID 14, CSF β -endorphin concentrations changed sporadically: four out of six infected animals showed increased values of 58.8 ± 5.4 pg/ml, and two other animals demonstrated extremely high concentrations of β -endorphin, reaching 230 and 301 pg/ml. Mean and diastolic blood pressures of Pichinde virus-infected animals were significantly decreased on PID 12 and 14, without marked changes in systolic pressure and heart rate (Table I).

After intravenous injection of β -endorphin (10 $\mu\text{g/kg}$) followed by constant infusion of β -endorphin (15 $\mu\text{g/kg}\cdot\text{hr}$) for 1 hr, plasma β -endorphin concentrations

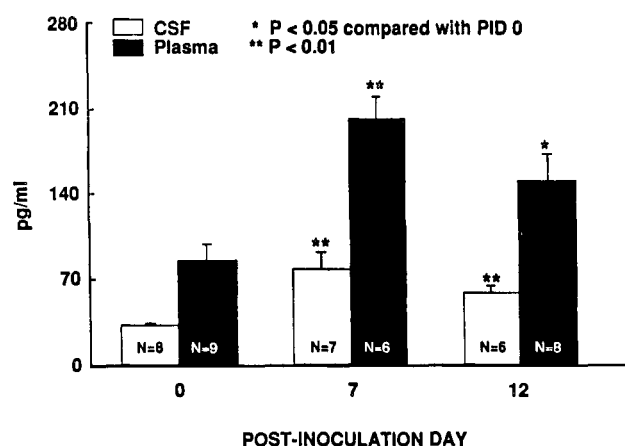


Figure 1. Increases of plasma and CSF β -endorphin concentrations during Pichinde viral infection in strain 13 guinea pigs.

Table I. Effects of Pichinde Viral Infection on Arterial Blood Pressures and Heart Rate in Anesthetized Strain 13 Guinea Pigs^a

	Control (n = 19)	PID 7 (n = 15)	PID 12 (n = 14)	PID 14 (n = 6)
Blood pressures (mm Hg)				
Systolic	76 $\pm$ 4	76 $\pm$ 6	67 $\pm$ 7	65 $\pm$ 8
Diastolic	55 $\pm$ 2	50 $\pm$ 3	36 $\pm$ 3 ^b	32 $\pm$ 2 ^b
Mean	62 $\pm$ 3	60 $\pm$ 4	49 $\pm$ 4 ^c	46 $\pm$ 5 ^c
Heart rate (beats/min)	215 $\pm$ 3	224 $\pm$ 9	196 $\pm$ 13	195 $\pm$ 15

^a Data are mean $\pm$ SE.

^b $P < 0.01$.

^c $P < 0.05$ compared with control.

of the two control guinea pigs increased to >7000 pg/ml from the baseline value (84.2 ± 13.2 pg/ml). Under the same experimental conditions, CSF β -endorphin concentrations only increased to 135 and 202 pg/ml from the baseline value (30.8 ± 1.9 pg/ml). Mean blood pressures decreased 5–15 mm Hg from baseline values (56 and 60 mm Hg) by 10 min after β -endorphin injection, and systolic and diastolic blood pressures decreased simultaneously. Heart rate did not change at this dose. When doses of primer injection and constant infusion of β -endorphin were increased to 15–30 $\mu\text{g/kg}$ and 45–90 $\mu\text{g/kg}\cdot\text{hr}$, respectively, into two other control guinea pigs, both mean blood pressure and heart rate decreased markedly (Fig. 2). When doses of primer and constant infusion of β -endorphin were decreased to 5 $\mu\text{g/kg}$ and 5 $\mu\text{g/kg}\cdot\text{hr}$, respectively, little or no changes in blood pressure and heart rate were observed.

Discussion

Pituitary gland, peripheral tissues (21, 22), and immunocytes of the immune system (23) secrete β -endorphin into the circulation. We demonstrated that β -endorphin concentration in CSF was 2- to 3-fold lower than that in plasma of control strain 13 guinea pigs, which suggests that circulating β -endorphin can hardly enter the brain ventricles (10). Released from neurons as a neurotransmitter, β -endorphin is partially destroyed at the synapses and in the interstitial fluid of the brain (24). These destructive sites may also explain why the β -endorphin concentration in CSF was lower than in the plasma of control guinea pigs. Hamel (25) found that CSF β -endorphin concentrations in man were significantly lower than in plasma. These results support our findings of a lower β -endorphin concentration in CSF as compared with plasma of guinea pigs. In contrast, however, De Riu *et al.* (11) and Jeffcoate *et al.* (26) showed that β -endorphin concentrations were higher in CSF than in plasma of dogs and man.

The intricate association between the immune and neuroendocrine systems through opioid pathways has

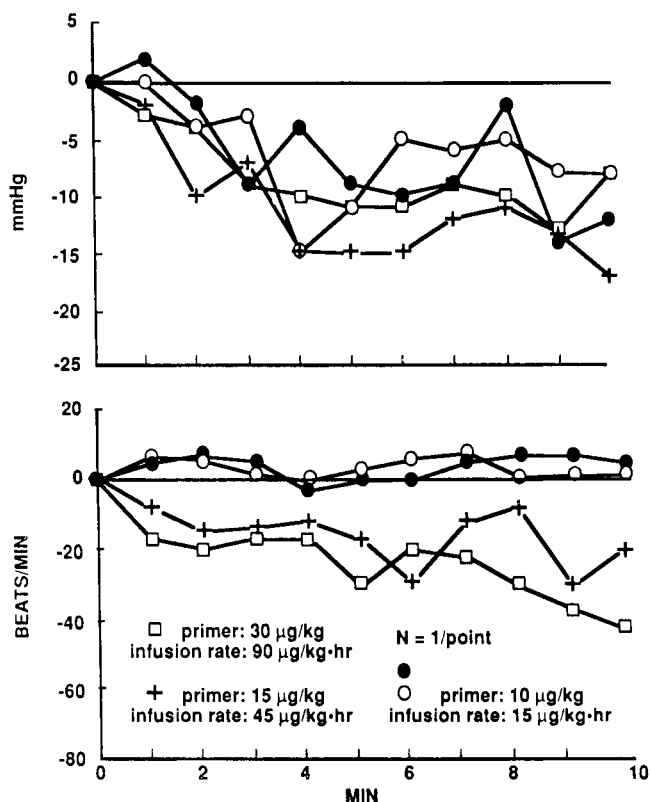


Figure 2. Changes in mean arterial blood pressure and heart rate after intravenous β -endorphin infusion in anesthetized, normal strain 13 guinea pigs.

been proposed for the past 10–15 years (23), and opioid-induced immunosuppression has been demonstrated (27). Increased endogenous β -endorphin concentration is also associated with stress (10, 11). Holaday (28) demonstrated that plasma β -endorphin levels increased 5- to 10-fold during endotoxemic shock in monkeys. Wright and Weller (29) observed that naloxone reversed leukopenia, thrombopenia, and hypothermia of mice during endotoxemic shock. Furthermore, Faden and co-workers (12–14) found that naloxone attenuated hypotension of endotoxemic, hemorrhagic, and spinal neurogenic shock, and prolonged survival. The pressor effects of naloxone might be the result of blockade of opiate receptors in the cardiovascular center of medulla oblongata (28).

β -Endorphin is involved in thermoregulation of the central nervous system (30, 31). In rats, intracerebroventricular injection of small doses (30–1000 ng/rat) of β -endorphin produced an increase in body temperature, whereas larger doses (3–10 μ g/rat) caused a decrease in body temperature (30). By blocking opiate receptor with naloxone, both hyperthermia and hypothermia induced by opioid agonists were attenuated (31).

Pichinde viral infection produces fever, leukopenia, thrombopenia, inalterness, terminal hypothermia, and hypotension (1–4). We also demonstrated that naloxone prolonged survival of Pichinde virus-infected ani-

mals (16), which suggests a possible involvement of β -endorphin during Pichinde virus infection. However, plasma and CSF β -endorphin concentrations were not measured in these previous experiments.

A single intracisternal administration of β -endorphin (3.1–6.9 ng/rat) caused bradycardia and hypotension in rats (6). Appel *et al.* (32) injected β -endorphin (200 pg/rat) into the cisterna magna of the adult rats; hyperglycemia and increased concentrations of plasma epinephrine, norepinephrine, and dopamine were observed. The increased plasma catecholamine might be a result of stimulated baroreceptors during endorphin-induced hypotension. Because total CSF volume of the rat is estimated to be 0.4–0.5 ml (33), CSF β -endorphin concentrations of these β -endorphin-injected rats could reach 400–500 pg/ml, which is about 5-fold higher than CSF β -endorphin concentrations in our infected guinea pigs. In fact, hyperglycemia develops in Pichinde virus-infected animals (2).

In the present study, we infused β -endorphin intravenously after a priming dose to the control guinea pig. The reason for injecting a primer was to achieve an elevated plasma β -endorphin more quickly to exert a clear cardiovascular response. When CSF β -endorphin concentrations of the β -endorphin-infused control guinea pig reached about 1.7- to 2.6-fold higher than the mean CSF β -endorphin value in the Pichinde virus-infected animals on PID 7, hypotension developed without significant changes in heart rate.

Brain β -endorphin may inhibit cardiovascular functions by acting on the medulla oblongata (6, 7). Circulating β -endorphin may have limited access to the brain via circumventricular sites, such as the area postrema, which lacks a blood-brain barrier. The area postrema is densely populated with opiate receptors that modulate cardiovascular functions (28). Therefore, a slight increase in CSF β -endorphin may exert a greater influence on the cardiovascular system, as compared with severe hyperendorphinemia.

During Pichinde virus infection, Jahrling *et al.* (3) showed that the brain was one of the major target organs with high contents of virus. Highly infected brain may be associated with unique biochemical changes in the central nervous system. In this study, we demonstrated that Pichinde viral infection caused increased β -endorphin concentrations in both CSF and plasma of strain 13 guinea pigs. The peak concentration was observed on PID 7. Previous studies in strain 13 guinea pigs showed that Pichinde viral infection caused a decrease in cardiac output by PID 7 without significant changes in arterial blood pressure (2).

The discrepancy of blood pressure changes during Pichinde virus infection and after β -endorphin intravenous infusion indicates that these two conditions are not equal. During Pichinde virus infection, not only do β -endorphin concentrations increase in plasma and

CSF, but also other opioids, including met-enkephalin, may be produced from the adrenal medulla (34) and brain (35). In fact, more platelet-activating factor (36), leukotrienes (1, 2), atrial natriuretic peptide (37), serotonin, and catecholamines (38) are known to be released in Pichinde virus-infected guinea pigs. Furthermore, indirect evidence suggests that prostaglandins may be involved, since aspirin can also prolong survival after Pichinde virus inoculation (16). All of these results suggest that physiological stress (10, 11, 39) can result from Pichinde virus infection. We postulated that cellular metabolic deficiencies and multiorgan dysfunction of Pichinde virus-infected guinea pigs (1–5) may be associated with adverse activities of several released chemical mediators.

Although the demonstrated chemical substances or mediators produce certain adverse cardiovascular and pulmonary reactions (6–9, 40–45), the overall results are that the arterial blood pressures are well maintained during the major course of Pichinde virus infection. The unchanged blood pressure is due to increased total peripheral resistance, despite drastic decreases in cardiac output and other cardiac functions (2, 4, 5). Thus, we suggest that increased concentrations of β -endorphin in CSF and plasma may play a role in the pathophysiological mechanism, and β -endorphin may be partially responsible for producing some of the cardiovascular disturbances during Pichinde virus infection.

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